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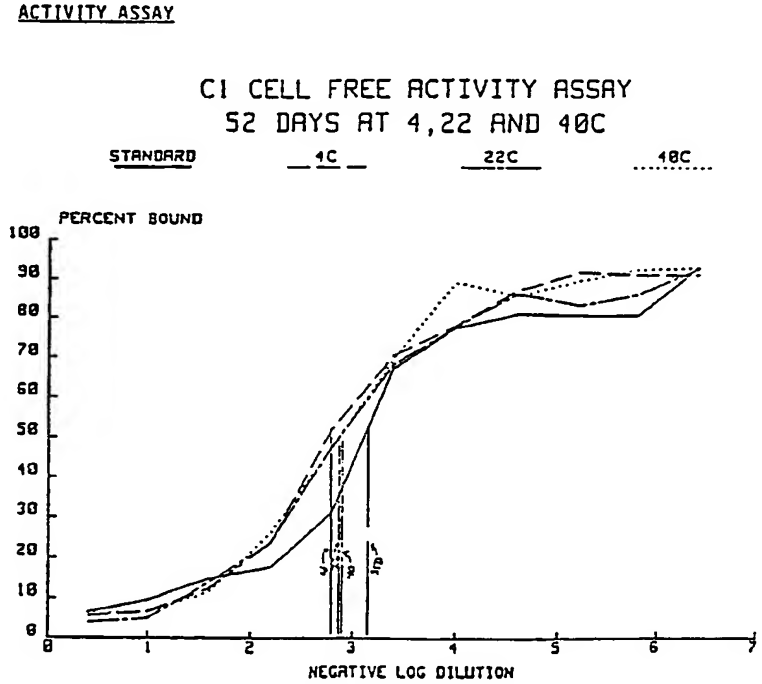

(57) Abstract

A lyophilized formulation for the stabilization of mouse monoclonal antibodies intended for parenteral administration containing a low pH buffer, a stabilizing carbohydrate and monoclonal antibodies. Lyophilization results in a dried, stable product which is readily reconstituted to provide an injectable, particle-free antibody solution which can be administered without prior filtration.

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FREEZE-DRIED FORMULATION FOR ANTIBODY PRODUCTS

Background of the Invention

A major challenge that exists in the field of new protein drugs is in the formulation of protein solution products that maintain both protein solubility and biological activity. It is well known that purified monoclonal antibodies, for example, tend to precipitate from the solution when subjected to physical or chemical stress, or over time. Many protein preparations are unstable in solution, and the instability is manifested in the formation of insoluble particles in the solution. The formation of these particles is often increased by physical stress, such as storage for prolonged periods and shipping; and by chemical stress such as dilution or mixing with an additive. Such particulates are not acceptable in terms of visual appearance of the product or USP specifications, and cause much difficulty in the development of suitable liquid formulations of monoclonal antibodies intended for intravenous injection.

The precipitation of proteins from aqueous solutions is a well known phenomenon. Immunoglobulins, in particular, possess characteristics that tend to generate particles in solution, so that an immunoglobulin solution generally must be filtered before used for intravenous injection.

Conditions which lead to increased precipitation of immunoglobulins include long term storage, filling vials with automated equipment and shipping.

Various methods for stabilizing protein preparations have been used, including increasing the concentration of protein in solution or adding an additional protein, such as human serum albumin. However, such preparations may not be acceptable for therapeutic purposes.

Various carbohydrates are known to stabilize and/or enhance the stability of certain biologically active protein preparations. For example, U.S. Patent 4,186,192 to Lundblad et al. discloses the use of maltose to increase the stability of an immune serum globulin preparation for intramuscular or intravenous administration. T. Arakawa and S. N. Timasheff describe the stabilization of protein structure by lactose and glucose in Biochemistry, 21:6536-6544 (1982). Gazzei et al. describe the addition of saccharose to a formulation of immunoglobulins in European Patent Application 221,505.

Maltose has been added to protein solutions to enhance stability of the proteins in solutions and to impart isotonicity to a solution. Maltose has many advantages, including availability in pure form and stability in aqueous solutions. Preparations containing maltose can be autoclaved without browning of the solution. Maltose in small quantities is practically physiologically inert. When administered parenterally, it is gradually

converted to glucose by the enzyme maltase; however, the conversion to glucose is gradual and frequently undetectable when serum glucose is measured. Therefore, there is no detectable increase in circulating insulin levels. In U.S. Patent 4,449,073, Tenold discloses the use of maltose in a preparation of immune serum golubin to import physiologically acceptable isotonicity to the preparation. Tenold specifies 10% weight to volume maltose for this purpose. Lundblad et al., report in U.S. Patent 4,186,192 that the stability of a solution of immune serum globulin is increased by adding maltose in a concentration between 5 and 18% by weight. Fernandes et al. describes a preparation of intravenous gamma globulin stabilized with maltose to minimize precipitation and improve in vitro shelf stability in Vox Sang, 39:101-112 (1980).

One approach used to circumvent this problem is the development of lyophilized drug forms. Lyophilization is the freeze-drying of biological material. Generally, the material is first frozen and then placed in a high vacuum so that the water (in the form of ice) sublimes into the vacuum and the non-water components are left behind in an undamaged state. The resulting product is a cake-like substance which can then be stored for long periods of time without degradation. The powder is reconstituted at the time of use to a liquid product by the addition of water, saline or other diluent. The reconstituted solution is then ready to be administered to the subject.

Summary of the Invention

This invention relates to a lyophilized composition containing a monoclonal antibody, a buffer and maltose, intended for parenteral administration. The invention further relates to a method for preparing the lyophilized product. The lyophilized composition of the invention preserves the biological activity of the monoclonal antibody, and minimizes formation of particulates caused by precipitation or aggregation of the monoclonal antibody when the freeze-dried product is reconstituted. The reconstituted solutions are particle-free, show no loss of biological activity and can be administered without prior filtration.

Brief Description of the Drawings

Figure 1 shows the gel filtration HPLC results comparing lyophilized and liquid samples of IgG exposed to a range of stress temperatures for 95 days.

Figure 2 shows the results of an immunoreactivity assay comparing binding ability of both lyophilized and 10% maltose liquid samples of IgG which have been exposed to a range of stress temperatures for 95 days.

Figure 3 shows the HPLC gel filtration results of lyophilized 2% maltose samples exposed to a range of stress temperatures for 52 days.

Figure 4 shows the results of an immuno-reactivity assay for lyophilized samples of 2% maltose exposed to a range of stress temperatures for 52 days.

Detailed Description of the Invention

The formulation comprises a lyophilized composition containing a low pH buffer, a carbohydrate component and an antibody or antibody fragment. The liquid composition is prepared, then lyophilized to form a cake-like product. The lyophilized product can be stored for prolonged periods of time, and at elevated temperatures, without loss of biological activity, and is readily

reconstituted into a particle-free solution by the addition of water, saline or other diluent.

Lyophilization is a freeze drying process which is often used in the preparation of pharmaceutical products to preserve their biological activity. The process generally involves drying a previously frozen sample in a vacuum to remove the ice, leaving the non-water components intact, in the form of a powdery or cake-like substance. The dried product is then be reconstituted at the time of use by the addition of water, saline solution, buffer or other appropriate liquid to produce a solution of the antibody. The advantage of lyophilization is that the biological activity of the antibody is preserved, and solution stress caused by exposure to varying temperatures, shear forces and shipping is avoided. The lyophilized product is readily reconstituted and is particle free, so it can be administered intravenously without prior filtration.

One embodiment of the present formulation is a solution containing a buffer, maltose and a murine monoclonal antibody or antibody fragments, such as immunoglobulin G (IgG), which is lyophilized to form a cake-like product.

All subclasses of immunoglobulin G can be used, e.g. immunoglobulin G_1, G_2, G_{2a}, G_{2b} and G_3. The antibody or antibody fragment can be derivatized so that it binds with a radiometal, such as Technetium-99m or Indium-111. The antibody can be derivatized either by changing its native state so that a radiometal can be bound directly to the

protein, or with a chelating agent such as diethyl-enetriaminepentaacetic acid (DTPA). The derivatized antibody can be used as a radio pharmaceutical due to the derivatized antibody's ability to bind a radioactive heavy metal (e.g. Indium-111 or Technetium-99m) to the antibody protein, forming a protein-radiometal complex, or, in the case of a chelating agent, a protein-chelate-radiometal complex. The radiolabeled antibody can then be used in immunoscintigraphy, for example, for in vivo diagnostic imaging of tumors or disease sites. IgG labeled with a radiation source, such as Tc-99m, can be administered to a subject. The labeled antibody localizes at the site defined by anti-IgG, and the site can then be scanned with a gamma camera, and an image obtained.

A preferred embodiment of the invention is a formulation containing a buffer having a low pH (between about 3 to 6), maltose and IgG. Preferred buffers include sodium acetate, phosphate and citrate buffers. A more preferred embodiment is a formulation containing about 5-100 mM sodium acetate, having a pH between about 3-6, 2-10% maltose and 1 to 25 mg/ml IgG.

The liquid formulation can be lyophilized using appropriate drying parameters. The following drying parameters are preferred: a primary drying phase

temperature of about -40°C to -42°C and pressure between about 50 mTorr to about 80 mTorr; and a secondary drying phase at ambient temperature, and pressure between about 50 mTorr to 80 mTorr.

Maltose, which is used to stabilize the antibody solutions, is described in detail in, for example, the Merck Index, 10th edition, Merck and Co., Inc. Rohway, NJ (1983). Maltose is a dissaccharide (4-O--D-glucopyranosyl-D-glucopyranose) having the general formula $C_{12}H_{22}O_{11}$, which has been established as useful for stabilizing immunoglobulin solutions. (See U.S. Patent 4,499,073 to Tenold and U.S. Patent 4,186,192 to Lundblad, et al.). Maltose is not metabolized by humans when administered intravenously, and is excreted as maltose with no apparent elevation in blood glucose levels or release of insulin.

The lyophilized product is redissolved at the time of use in a diluent (e.g., sterile water or saline) and yields a particle-free solution. Reconstitution is complete in about two minutes. The reconstituted antibody solution is particle-free even after prolonged storage of the lyophilized cake at 37°C. The reconstituted solution is then administered parenterally, preferably intravenously, to the subject.

The invention is further illustrated by the following exemplification:

Exemplification

Example 1. Preparation of a Stabilized Lyophilized Formulation of Immunoglobulin G.

Desalting of IgG from 0.9% Saline Into a solution of 10mM Sodium Acetate (pH 4.5) containing 10% Maltose

A 3.3 L Sephadex G-25 medium column (Pharmacia) packed in 0.1 M sodium hydroxide was equilibrated with a solution of 10 mM sodium acetate (pH 4.5) and 10% w/v maltose before applying the protein. 500 ml of IgG (Centocor, Inc., Malvern, PA) at a concentration of 11.54 mg/ml (5.693g total) was applied to the column at a flow rate of 550 ml/hr. The protein peak was collected and concentrated to 10 mg/ml via stirred cell with YM30-membrane (Amicon).

The entire lot was split in half, and one part was maintained in the liquid state, and the other dedicated for freeze-drying. Each part consisted of 25 vials that were subjected to temperature stress testing upon the completion of the lyophilization process.

Lyophilization of IgG Formulation

Twenty milliliter vials (West Co.) were filled with 10 ml of the formulation and placed in the freeze dryer (FTS). Since freeze-drying was not under sterile conditions, 100 ul of 10% sodium azide was added to the lyophilized samples as a baceterio-static agent.

Placebo vials, filled with the identical buffer and maltose but lacking antibody, were also placed

in the lyophilizer. The degree of supercooling of the formulation was -6°C and formed a uniform matrix. The product was initially frozen to -45°C. Ater freezing to this temperature, the shelf surface temperature was adjusted to between -40 to -42°C, with the pressure reduced to 50 mTorr to initiate the primary drying phase.

Once the primary drying phase was completed, the shelf surface temperature was raised to +20°C to initiate the secondary drying phase. The composition of the gases in the chamber were monitored via residual gas mass spectrum until nitrogen had replaced water vapor as the species in the chamber. The secondary drying phase required about 8 hours.

After reaching this point, the chamber was back-filled with dry nitrogen and the vials were stoppered.

The procedure yielded 25 vials of lyophilized product at 10 ml/vial, 10.0 mg/ml.

Temperature Stress Study

Representative vials from both the liquid and freeze-dried formulations were exposed to temperatures of 4, 22, 37 and 50°C.

After 95 days under such conditions, one vial from each formulation at each temperature was removed for analysis. The lyophilized product was reconstituted with 10 ml of water. The reconstitution times are shown in Table 1 below.

Table 1

Reconstitution Times (Lyophilized Product)

Sample	Reconstitution Time (Minutes)
t_o sample	1
95 day samples	
4°C	2
22°C	2
37°C	2.5
50°C	2

Each vial was then visually examined for particulates. The vials were examined by holding the vial directly in front of a black background and lighting the vial from below. Visual comparison of reconstituted lyophilized product to liquid samples demonstrated the superiority of the lyophilized product. The liquid samples all contained particulation of some degree, whereas the reconstituted samples were clear and free of particulates. The liquid samples also showed increased yellowing as the temperature increased. The results of the visual examination are shown in Table 2.

Table 2

Visual Examination

T°C	Formulation	Observation
4	liquid	small flakes
22	liquid	small flakes and fine precipitation
37	liquid	fine particulates, slightly yellow liquid
50	liquid	moderate particulation, yellow liquid
4	lyophilized	clear
22	lyophilized	clear
37	lyophilized	clear
50	lyophilized	clear

Liquidborne Particle Analysis

The vials were then analyzed in a liquidborne particle analyzer (Climet). The samples were analyzed for particles ranging in size from 5 to 50 microns. Subvisible liquidborn particle analysis showed some superiority of the reconstituted lyophilized samples over the liquid samples. The results are shown in Table 3.

Table 3

Subvisible Liquidborne Particle Analysis
(particles per milliliter)

		Differential					Total					
T°C	Formulation	5u	10u	20u	25u	30u	50u	5u	10u	20u	25u	30u	50u
4	Liquid	188	92	18	12	50	172	532	344	242	234	222	172
22	Liquid	182	50	6	2	6	0	246	64	14	8	6	0
37	Liquid	282	102	12	4	10	2	412	130	28	16	12	2
50	Liquid	204	92	14	6	10	4	330	126	34	20	14	4
4	Lyophilized	230	64	4	2	0	0	300	70	6	2	0	0
22	Lyophilized	236	42	4	2	2	0	256	50	8	4	2	0
37	Lyophilized	194	28	4	2	2	0	230	36	8	4	2	0
50	Lyophilized	550	82	14	10	8	0	664	114	32	18	8	0

HPLC Gel Filtration Chromatography

The samples were subjected to HPLC gel filtration chromatography. An HPLC unit (DuPont Zorbarx GF-250 HPLC) was equilibrated with .2 M sodium phosphate buffer (pH 6.8) at a flow rate of 1 ml/min. Absorbance wavelength was set at 214 nm. Undiluted samples were injected and analyzed. The results showed that both liquid and reconstituted samples were relatively stable under the 4°C and 22°C stress conditions. At 37°C and 50°C, the liquid samples showed a measurable increase in dimer concentration generating 10.48% dimer at 37°C and 90.05% dimer at 50°C. The reconstituted samples show no increase in dimer levels at 37°C and 50°C. The results are shown in Figure 1.

Immunoreactivity Assay

An assay was performed on the samples to determine the relative immunoreactivity of the IgG in the samples. The activity analysis showed that antibody activity was maintained in both liquid and lyophilized samples. The results are shown in Figure 2.

The procedure used for determining antibody immunoreactivity is as follows:

In a polyvinylchloride (PVC) plate labeled "Antigen", 50 ul of diluted antigen was dispensed into each of the 96 wells. The plate was placed uncovered at 37°C to air dry for four hours. After all the solution had evaporated from the wells, each well was washed twice with 100 ul of phosphate buffered saline (PBS). To each well, 200 ul of 5% bovine serum albumin (BSA) in PBS was dispensed, and allowed to incubate covered for 2 hours at room temperature.

In a separate PVC plate labeled "Antibody", 75 ul of 10% BSA in PBS was dispensed into wells in rows 2 through 12. 100 ul of the reconstituted lyophilized samples and standard antibody solutions that had been pre-diluted to 400 micrograms/ml were dispensed to wells in row 1. With a pipetting device, 25 ul was removed from row 1, dispensed to row 2 and mixed. This dispensing was repeated for the wells in rows 3 through 11. The final 25 ul increment was discarded. Row 12 was the 100% binding row.

PVC plates labeled "Antigen" were washed twice with 200 ul of PBS. Using new pipette tips each time, 50ul of the prediluted antibody was transferred from the PVC plate labeled "Antibody" to corresponding wells on the "Antigen" plate. To each well on the "Antigen" plate containing 50 ul of prediluted antibody, 20 nanograms of ^{125}I labeled antibody in 50 ul of 1% BSA in PBS was added. The plate was covered and incubated at 4°C for 20 hours. After 20 hours at 4°C, the excess radiolabel was washed from the plate with three washes of PBS, and counted in a gamma counter.

The data were analyzed by averaging duplicate counts. Counts per minute (cpm) versus the negative log of the cold antibody were plotted. From row 12 of each antibody dilution series, the number of cpm found, which represents 100% of binding, was calculated. This number was divided by two to obtain the 50% maximal binding value. The 50% maximal binding value for each antibody dilution series was compared to the point on the curve for each antibody titration representing the cpm of the 50% maximal point. The protein concentration was determined from this point.

SDS:PAGE Chromatography

The samples were subjected to SDS:PAGE chromatography to determine molecular integrity of the IgG after stressing at various temperatures. Non-reduced samples were prepared in a microfuge

tube. Protein sample, PBS, and SDS and coomassie stock were mixed to obtain a protein concentration of 2mg/ml. Fifty microliters (50 ul) of the protein solution was reduced by adding 5 ul of β-mercaptoethanol. The samples in tubes were exposed to boiling water for 2 minutes. Using a 1 ul applicator comb, each prepared sample was applied to the gel (Pharmacia Phast Gel SDS Page Gels (8-25%)). The gel was run according to the manufacturers instructions.

SDS:PAGE chromatography demonstrated the molecular integrity of the lyophilization material throughout the range of stress temperatures. The liquid formulation maintained its structure at 4°C and 22°C, but begins to show increasing fragmentation at 37°C and 50°C.

Native PAGE Chromatography

The samples were tested using Native PAGE chromatography. The samples were diluted with PBS (phosphate buffered saline), and applied to Native Page Gel (Pharmacia Phast Gel Native Page Gel 7.5% and 12.5% homogeneous). The gels were run, stained and preserved according to the manufacturer's instructions. Native PAGE results showed some alterations in the proteins exposed to different temperatures, for both reconstituted lyophilized samples and liquid samples. The proteins in the liquid samples become more negatively charged at higher temperatures. Increases in negativity were observable with liquid samples stored at 22°C and

37°C, with no protein entering the gel at 50°C indicating extensive protein denaturation. The reconstituted lyophilized samples were observable in the gel throughout the temperature range, and the lyophilized 50°C sample is less negative than the liquid 37°C sample. This phenomenon was consistent in both the 7.5% and 12.5% homogeneous gels, with the 12.5% gel appearing as more well-defined bands.

Isoelectric Focusing

Isoelectric focusing was conducted to determine any changes in the protein isoelectric point (pI) between the liquid and lyophilized samples. Samples were diluted with PBS and applied to an isoelectric fosucing gel (Pharmacia Phast Gel pH 3-9 Isoelectric Focusing Gel). The gel was run, fixed, stained and destained according to manufacturer's instructions. The results showed no alteration in the pI of the protein at any of the stress temperatures for the lyophilized product. The liquid samples demonstrated no alteration at 4°C and 22°C, but showed considerable movement toward the lower pH range at 37°C, and denaturation at 50°C.

Residual Moisture Analysis

Residual moisture analysis was performed on placebo lyophilized samples to determine the residual moisture attributable to the solution

without protein. Placebo vials contained a solution of 10 mM sodium acetate (pH 4.5), and 10% maltose. The placebo vials were lyophilized along with the vials containing antibody under the same conditions. For the residual moisture analysis, the contents of placebo vials were removed into a dry pre-tared weigh boat (VWR Scientific) in a glove bag which had been previously purged with dry, inert gas. The sample was weighed to obtain an initial weight of the placebo material. The weigh boat with the placebo material was then placed in a vacuum oven (VWR Scientific) at 60°C, and pressure in the vacuum oven was reduced for 24 hours. The weigh boats were then removed from the oven into a glove bag purged with dry nitrogen gas, and allowed to equilibrate to room temperature. The samples were then reweighed. The sequence was repeated until the samples were brought to constant weight (there were no more reductions in weight). The final constant weight was divided by the original weight to calculate the precent residual moisture. The results are shown in Table 4.

Table 4

Residual Moisture Analysis (lyophilized)

Vial#	Weigh Boat Tare (g)	Weigh Boat + Placebo	Placebo	Placebo After Drying	Loss In Drying	% Residual Moisture
1	1.57387	2.41015	0.83628	0.81698	0.01930	2.31
2	1.54695	2.42704	0.88009	0.86034	0.01975	2.24
3	1.58552	2.44362	0.85810	0.83867	0.01943	2.26

AVERAGE VALUE = 2.27%

Example 2. Preparation of a Lower Maltose Lyophilized Formulation for IgG

Solutions of immunoglobulin G were prepared and tested according to the procedures set out in Example 1, but reducing the amount of maltose in the formulation from 10% to 2%. This formulation consisted of 10mM sodium acetate (pH 4.5), 2% w/v maltose and IgG.

Lyophilization

Twenty milliliter vials were prepared and filled with 10 ml of the reduced-maltose formulation and placed in the freeze dryer. Placebo vials, filled with the identical solution of buffer and maltose, but lacking antibody, were also placed in the freeze dryer. Lyophilization was carried out as described in Example 1.

Temperature stress testing of the lyophilized product and the placebo vials was done by storing the test samples at 4, 22 and 40°C for 52 days. Some of the lyophilized samples were then reconstituted with 10 ml of water. The reconstitution times are shown in Table 5.

Table 5

Reconstitution Times (Lyophilized Product)

Sample	Reconstituion Time (Minutes)
t_o	0.5
52 day samples	
4°C	0.67
22°C	0.75
40°C	0.83

Visual examination of the vials for particulates was then performed. Visual examination showed no visible particulates in the reconstituted lyophilized samples. The results are shown in Table 6.

Table 6

Visual Examination

T°C	Formulation	Observation
4	lyophilized	Clear
22	lyophilized	Clear
40	lyophilized	Clear

Liquidborne Particle Analysis

Liquidborne particle analysis showed that all samples were within USP specifications for 10 and 25 micron-sized particles. The results are shown in Table 7.

Table 7

Subvisible Liquidborne Particle Analysis (52 Days)

	DIFFERENTIAL						TOTAL					
T°C	5u	10u	20u	25u	30u	50u	5u	10u	20u	25u	30u	50u
4	313	54	2	2	0	0	376	58	4	2	0	0
22	910	142	10	2	4	0	1063	158	16	6	4	0
40	676	108	4	2	2	0	794	118	10	6	4	0

HPLC Gel Filtration Chromatography

Gel filtration results showed that the reconstituted material stressed at 4°C and 22°C were identical with the starting material. At 40°C, however, some dimer formation was apparent (about 2.12%). The results are shown in Figure 3.

Immunoreactivity Assay

Immunoreactivity assay results indicate that all samples performed within specification for the

assay, and possessed activity comparable to the standard. The results are shown in Figure 4.

SDS:PAGE Chromatography

SDS-PAGE gels for all samples appeared the same when loaded at 2 mg/ml on the non-reduced and reduced gels. When samples were loaded at 1 mg/ml, some aggregated material was observed only in the 40°C sample.

Native Page Chromatography

Native:PAGE gels (both 7.5% and 12.5% homogenous) exhibited a slight alteration in surface charge in the 40°C stressed samples.

Isoelectric Focusing

Isoelectric focusing of the freeze dried samples showed no change in the banding patterns at any of the temperature conditions.

Residual Moisture

Residual moisture analysis performed on three of the placebo vials showed an average residual moisture value of 1.24%. The results are shown in Table 8.

Table 8

RESIDUAL MOISTURE ANALYSIS

VIAL #	WEIGH BOAT TARE (g)	WEIGH BOAT +PLACEBO	PLACEBO	PLACEBO AFTER DRYING	LOSS IN DRYING	% RESIDUAL MOISTURE
1	1.58839	1.77753	0.18914	0.18555	0.00359	1.90
2	1.54959	1.74645	0.19686	0.19471	0.00215	1.09
3	1.55476	1.71318	0.15482	0.15726	0.00116	0.73

AVERAGE VALUE = 1.24%

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

Claims

1. A monoclonal antibody composition comprising a lyophilized formulation of a buffer, a monoclonal antibody or antibody fragments, and maltose.

2. A composition of Claim 1 wherein the monoclonal antibody is immunoglobulin G.

3. A composition of Claim 2 wherein the amount of immunoglobulin G is between about 1 to about 25 mg/ml.

4. A composition of claim 1 wherein the antibody fragments are derived from immunoglobulin G.

5. A composition of Claim 1 wherein the antibody or antibody fragments are radiolabled.

6. A composition of Claim 1 wherein the amount of maltose is between about 2 to about 10 percent by weight.

7. A composition of Claim 1 wherein the buffer is selected from the group consisting of sodium acetate, phosphate and citrate buffer.

8. A composition of Claim 7 wherein the buffer is sodium acetate having a pH between about 3.0 and 6.0.

9. An immunoglobulin G composition for parenteral administration comprising a lyophilized formulation of:
   (a) sodium acetate
   (b) maltose
   (c) immunoglobulin G or a fragment thereof.

10. A composition of Claim 9 wherein the amount of sodium acetate is between about 5 to about 10 mM and has a pH of between about 3-6.

11. A composition of Claim 9 wherein the amount of immunoglobulin G is between about 1 and about 25 mg/ml.

12. A composition of Claim 9 wherein the immunoglobulin G comprises immunoglobulin G_{2a}.

13. A composition of claim 9 wherein the immunoglobulin G fragment is radiolabled.

14. A composition of Claim 9 wherein the amount of maltose is between about 2 to 10 percent by weight.

15. An improved immunoglobulin G composition containing sodium acetate, maltose and immunoglobulin G, wherein the improvement comprises lyophilizing the composition to form a lyophilized cake which may be reconstituted to yield an injectable solution of immunoglobulin G.

16. A method of preparing a monoclonal antibody formulation which comprises lyophilizing the formulation under conditions appropriate to form a lyophilized cake which is capable of being reconstituted into an injectable solution.

17. A method of Claim 16 wherein the formulation comprises sodium acetate, maltose and immunoglobulin G.

18. A method of Claim 17 wherein the formulation comprises:
    (a) about 5-10 mM sodium acetate having a pH of between about 3-6;
    (b) about 2-10 percent by weight maltose; and
    (c) about 1-25 mg/ml immunoglobulin G.

19. A method of Claim 16 wherein the lyophilization conditions comprise:
a primary drying phase wherein the temperature is between about -40°C to -42° C and the pressure is between about 50 mTorr - 80 mTorr; and a secondary drying phase wherein the temperature is ambient and the pressure is between about 50 mTorr - 80 mTorr.

20. A lyophilized cake comprising a stable composition of immunoglobulin G wherein the cake can be reconstituted to yield an immunoglobulin G

solution which has a particle count of less that about 150 particles of 10 micron size or greater per milliliter, and less than about 15 particles of 20 micron size or greater per milliliter.

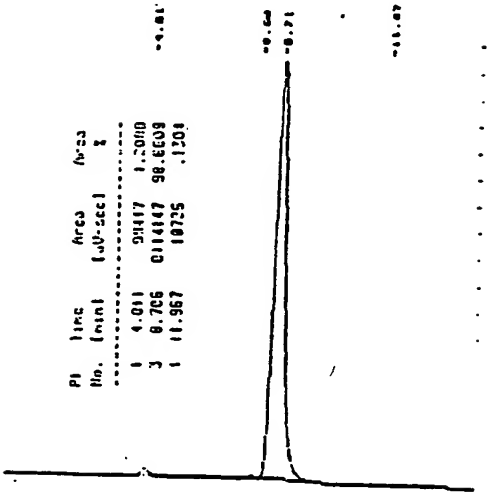
21. A lyophilized cake of Claim 20 wherein the cake is reconstituted with water or saline solution.

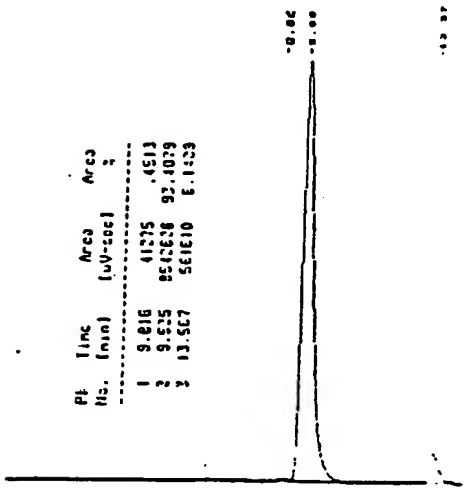
HPLC Gel Filtration

LIQUID

LYOPHILIZED

4°C

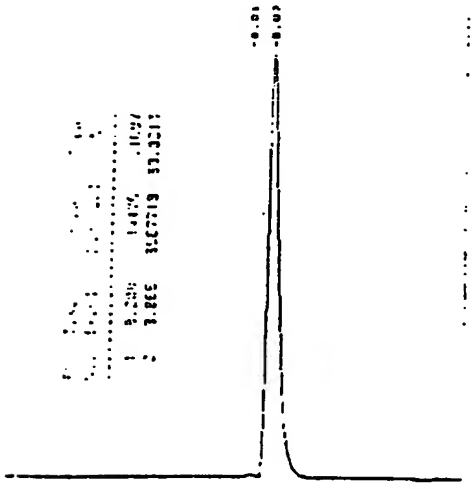




LIQUID

LYOPHILIZED

22°C



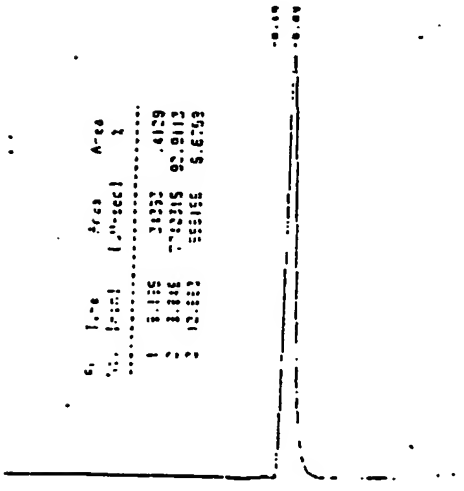


FIG. 1

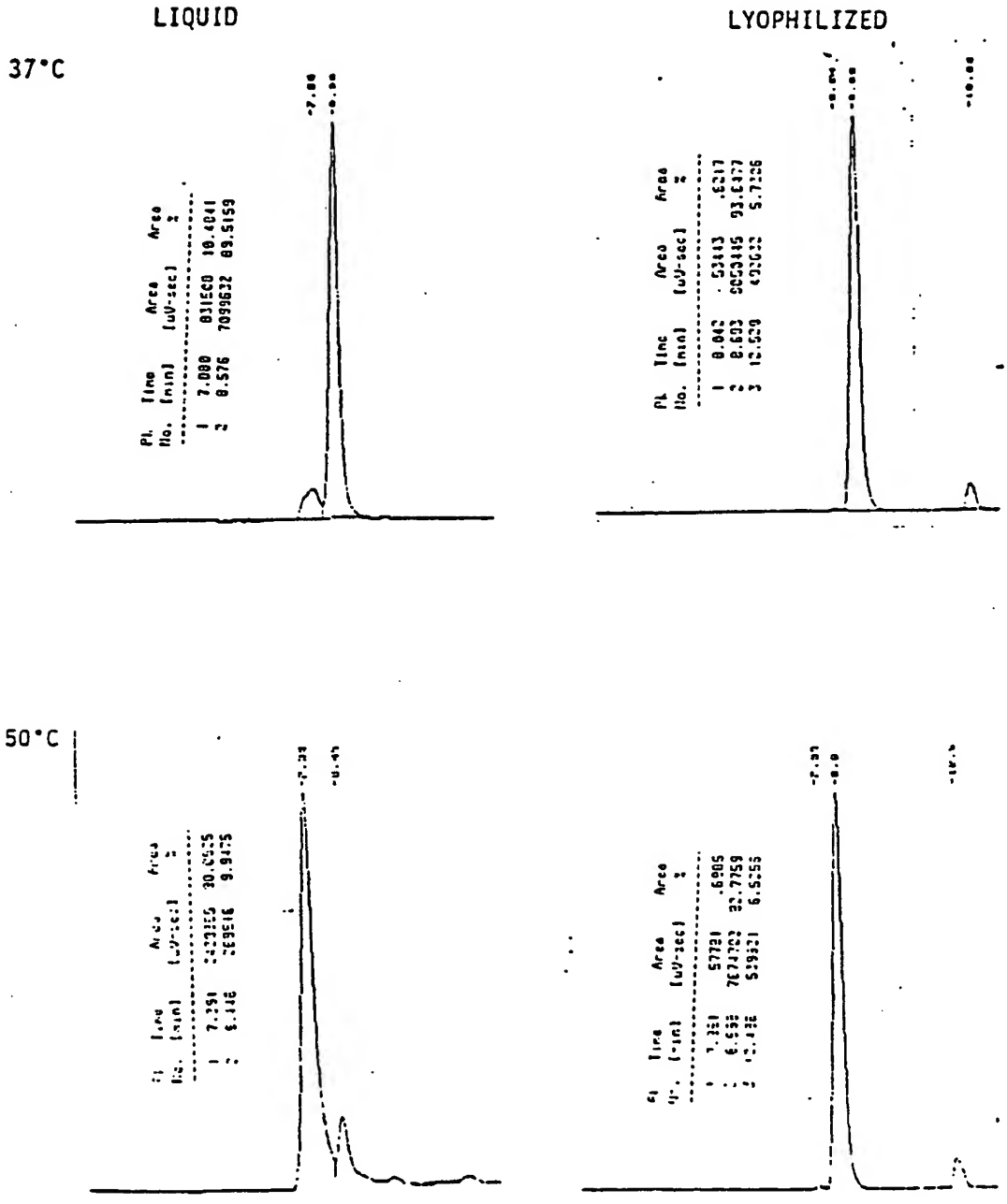


FIG. 1 (CONT.)

ACTIVITY ASSAY

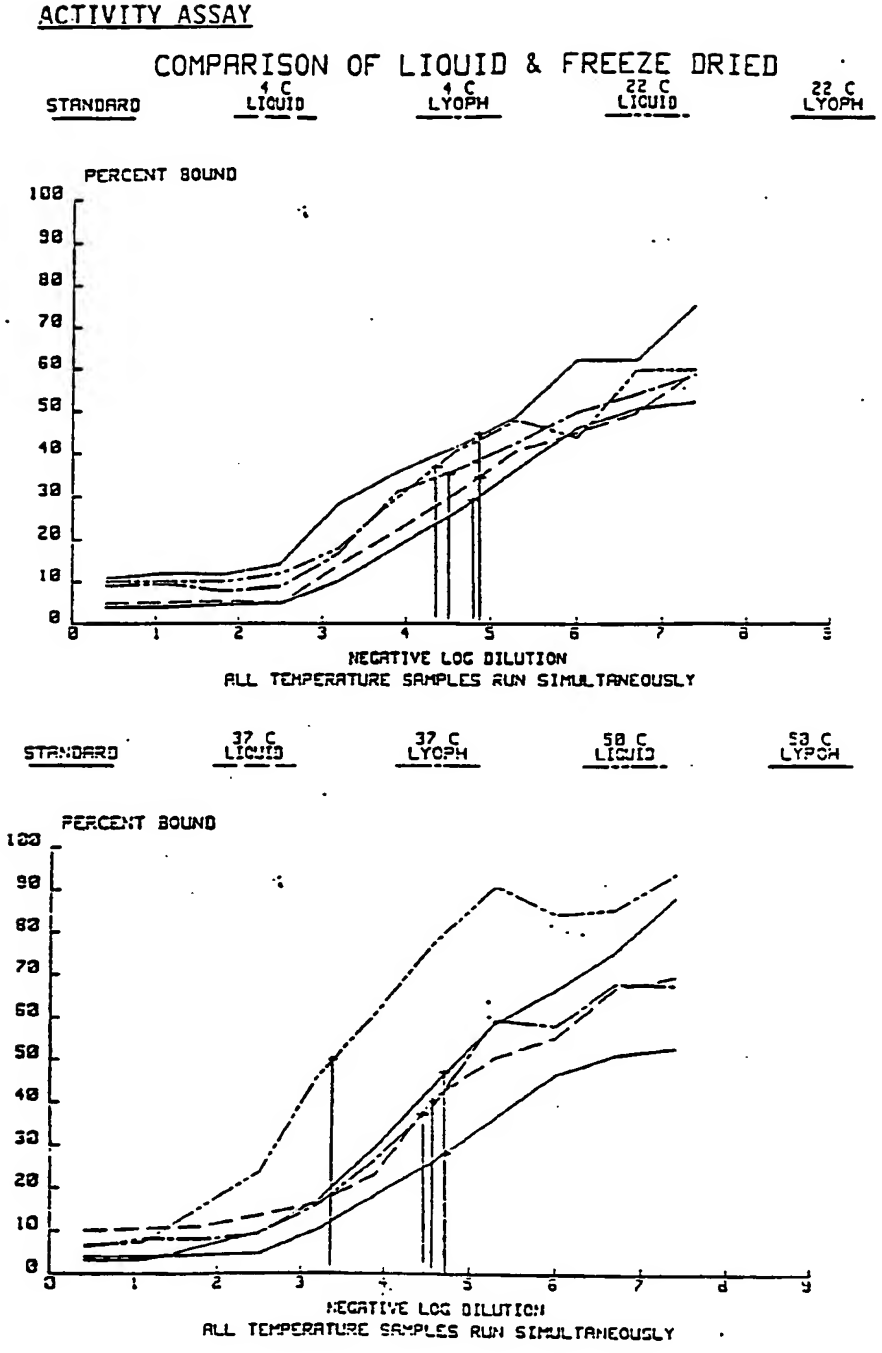


FIG. 2

HPLC Gel Filtration (52 Days)

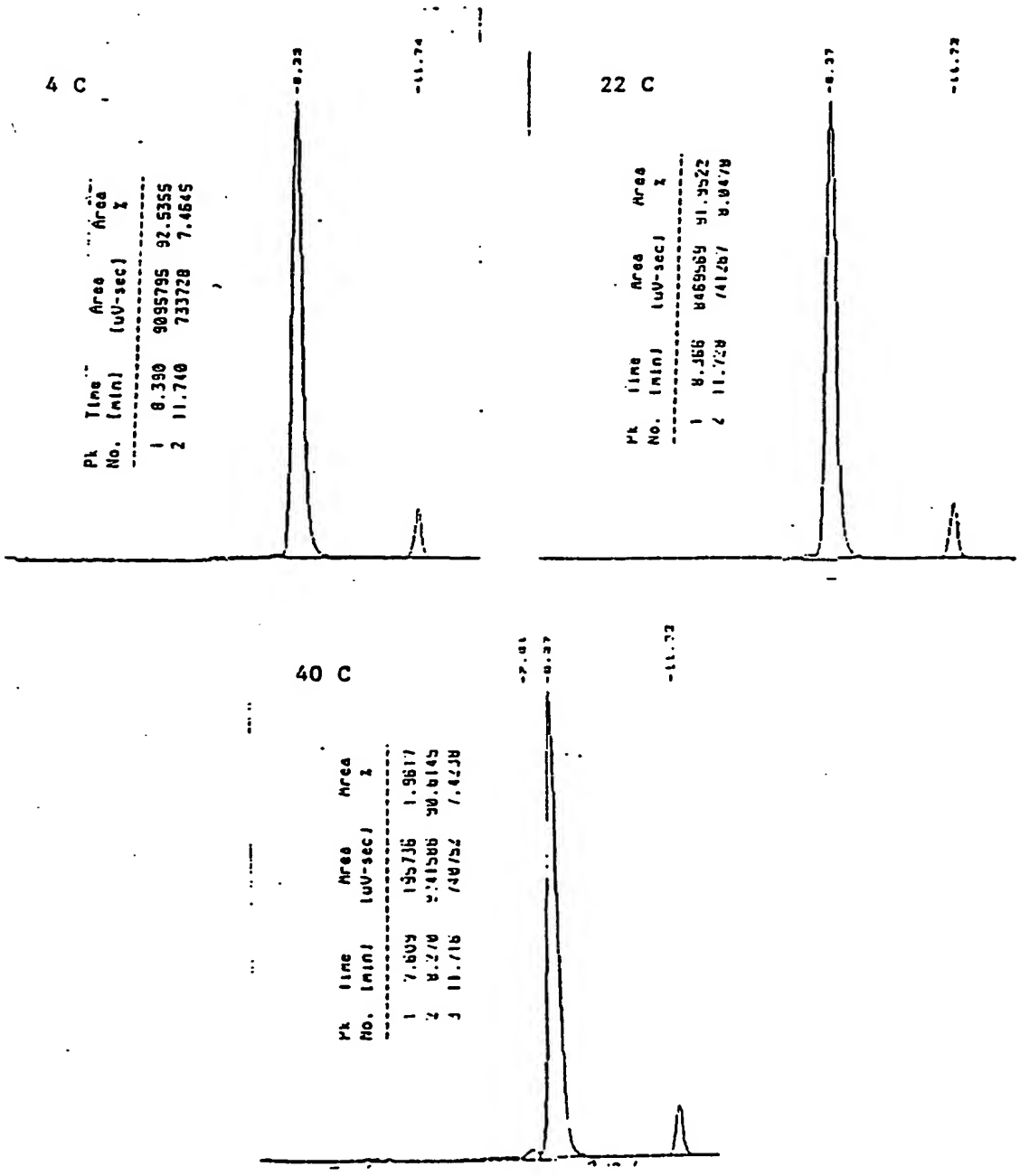


FIG. 3

ACTIVITY ASSAY

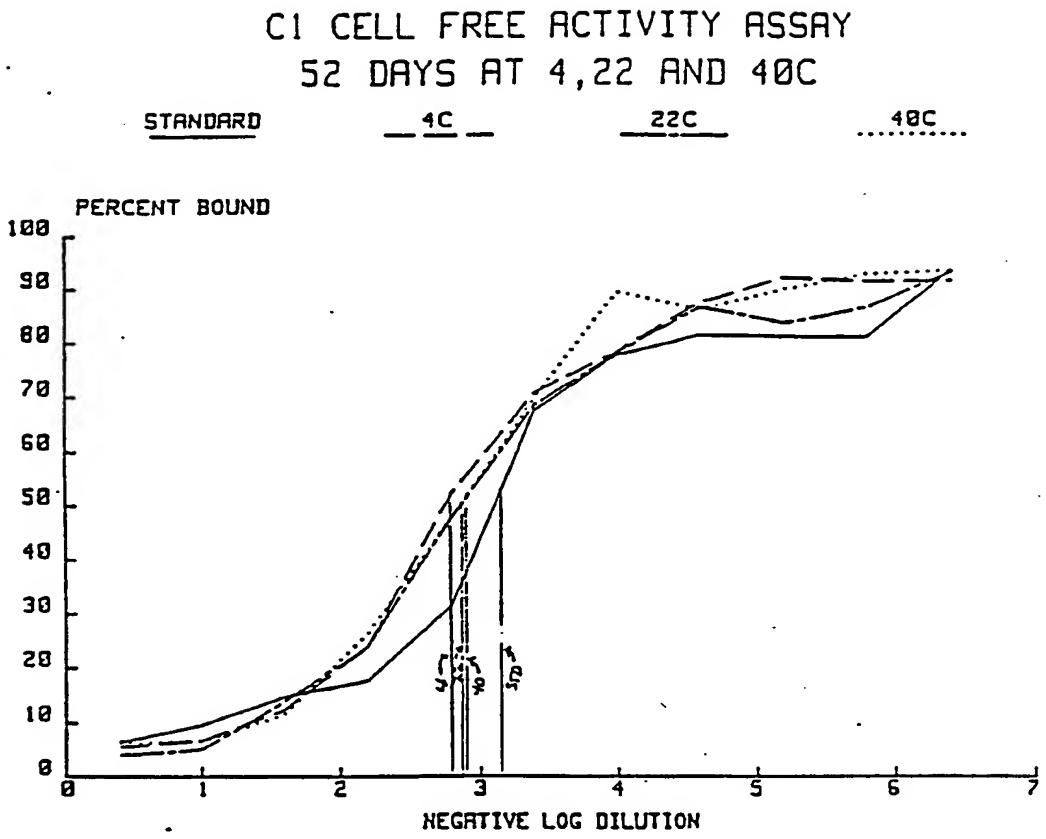


FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/02252

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 39/395, A 61 K 47/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	EP, A, 0073371 (CUTTER LABORATORIES, INC.) 9 March 1983 & US, A, 4499073 (cited in the application) --	
A	US, A, 4186192 (LUNDBLAD et al.) 29 January 1980 cited in the application	

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th September 1989	09. 10. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8902252 SA 29239

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 02/10/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0073371	09-03-83	US-A-	4396608	02-08-83
		AU-B-	549204	16-01-86
		AU-A-	8726582	03-03-83
		CA-A-	1183084	26-02-85
		JP-A-	58043914	14-03-83
		US-A-	4499073	12-02-85
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		CA-A-	1117417	02-02-82
		EP-A,B	0012156	25-06-80
		JP-A-	55083711	24-06-80